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Abstract

Optogenetics is an emerging biotechnology that combines genetics and optics to achieve precise optical control of specific cells in living organisms. Since the application of microbial opsin-based optogenetic strategies, optogenetics has made significant advances in the development and optimization of opsins, virus- and recombinase-based genetic targeting expression, and optical delivery technologies. Optogenetics is widely applied in modern neurobiology and plays a crucial role in the mechanistic investigation of neural circuits, behavior, central nervous system diseases, and psychiatric disorders. This article primarily reviews the developmental history of optogenetic technology, focusing on the optimization of optogenetic tools and targeted expression, aiming to provide a reference for research and development in optogenetics and related fields.

Full Text

Preamble

Research Progress of Optogenetic Techniques

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Abstract

Optogenetics is an emerging biotechnology that combines genetics and optics to achieve precise optical control of specific cells in living organisms. Since the application of microbial opsin-based optogenetic strategies, optogenetics has made significant advances in opsin development and optimization, genetic targeting expression based on viruses and recombinases, and optical transmission technologies. Optogenetics is widely applied in modern neurobiology, playing important roles in studying neural circuits, behavior, and the mechanisms of

central nervous system diseases and psychiatric disorders. This review primarily introduces the developmental history of optogenetic techniques, focusing on the optimization of optogenetic tools and targeted expression, aiming to provide references for research development in optogenetics and related fields.

Keywords: Optogenetic Techniques; Light Control; Opsin; Neurobiology

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Introduction

Optogenetics is an emerging biotechnology that combines optical and genetic techniques to achieve precise control of specific events in cells, tissues, and circuits [1]. The core components of optogenetics include photosensitive elements that respond to light stimulation and produce effects, as well as related technologies for recording and analyzing these activities, mainly covering: (1) expressing light-controlled elements in specific cells or tissues; (2) delivering light as a stimulus source to target cells or tissues; and (3) recording outputs and analyzing them [2]. As early as 1971, researchers discovered photosensitive proteins in bacteria, but they were not used to develop light-controlled elements at that time [3]. In 2005, microbial rhodopsin proteins were proven to be successfully expressed in specific neurons of mammals, endowing them with photosensitivity and thereby causing activation effects [4]. In the subsequent decade, with the demand for single-component light-controlled elements in the field of neurobiology, optogenetic technology developed rapidly and was widely applied in neurobiology research, such as mechanistic studies of neuropsychiatric diseases represented by Parkinson' s disease, epilepsy, and autism [5][6]. This article elaborates on the history of optogenetic technology development and focuses on introducing the optimization achievements of microbial opsins in optogenetic technology, the advantages and disadvantages of different genetic expression strategies, as well as existing limitations and future development directions, aiming to provide references for the development of related fields through a brief overview of recent research progress in optogenetic technology.

In 1979, Crick F proposed that the major challenge facing neurobiology was how to specifically control the activity of a particular type of neuron to study the relationship between neurons, neural circuits, and behavior. Due to the lack of cell specificity in electrical stimulation and the slow, temporally imprecise nature of drug effects, Crick F subsequently proposed that light might serve as a control means, providing the initial conceptual basis for the emergence of optogenetics [7].

Even before 1979, microbiologists had discovered that certain microorganisms

could express single-component photosensitive ion channel proteins. In 1971, Stoeckenius D and Oesterhelt S demonstrated that bacteriorhodopsin proteins could be activated under visible light conditions and function as channel proteins to achieve transmembrane ion transport [3]. Subsequently, in 1977, Matsuno-Yagi A and Mukohata Y discovered more members of this family [8]. In 2002, Hegeman P et al. further discovered channelrhodopsin (ChR) [9]. However, because microbial rhodopsin activation required the participation of chemical cofactors (all-trans retinal), researchers considered it an unfeasible strategy to use microbial rhodopsins for optical control in mammalian neurons [10].

Consequently, researchers attempted to achieve neuronal photoactivation through multi-component photosensitive proteins or photosensitive small molecules combined with ion channels. In 2002, Zemelman B et al. developed a multi-component photoactivation strategy named "chARGe" using Drosophila photoreceptor-related genes, including rhodopsin, -arrestin-2, and G protein subunits [11], and with the help of gene expression technology, first achieved the activation of a small subset of specific neurons within a mixed population using light [12][13]. Similarly, Banghart M et al. achieved precise and reversible optical control of neuronal activity in rats by coupling photosensitive chemical molecules to potassium ion channels [14]. However, technical limitations in multi-component protein expression, chemical modification of proteins, and tissue penetration of chemical small molecules hindered further application of multi-component optogenetics.

Until 2005, Boyden S et al. first successfully expressed microbial rhodopsin proteins in mammalian neurons and achieved neuronal photoactivation without requiring any auxiliary factors or components. Subsequent research confirmed that mammalian cells naturally contain the necessary cofactor all-trans retinal for activating bacterial rhodopsins [4]. In the following years, similar discoveries were reported successively. By 2010, various microbial rhodopsin proteins, including channelrhodopsin (ChR), bacterial rhodopsin, and halorhodopsin, had been proven capable of optically activating or inhibiting mammalian neurons, thereby enabling optogenetic control in intact mammalian brain tissue and even freely moving mammals [15][16]. This opened the door to single-component optogenetics.

2 Advances in Optogenetic Technology

After microbial rhodopsin proteins were proven useful for activating neurons, researchers further discovered naturally occurring photosensitive proteins with different characteristics in organisms, named opsins. Among them, opsins found in microorganisms (Type I) are mostly ion channels and ion pumps, while opsins in vertebrates (Type II) are mostly G protein-coupled receptors [17][10]. Due to the faster kinetic properties and single-component genetic manipulation advantages of microbial opsins [10], further development of optogenetic technology has mainly focused on the optimization and modification of microbial opsins, targeted expression of opsins, and development of optical transmission technolo-

gies.

2.1.1 Photoactivatable Opsins

Channelrhodopsin (ChR) is an ion channel found in single-celled algae, among which ChR2 (a subtype of ChR) is the most widely used [9]. ChR2 is a non-selective cation channel that opens under blue light (420 nm), causing neuronal depolarization and activation, and in 2005 successfully achieved optical control of neuronal activation in cultured mouse hippocampal cells [4][18].

Meanwhile, through site-directed mutagenesis and chimeric modification of ChR2, opsins with different kinetic characteristics have been further developed to meet applications under various research conditions [10]. Ultrafast opsins are ChR2 mutants with high-frequency activation and rapid inactivation properties, mainly including ChETA and ChIEF [19][20]. Ultrafast opsins can activate neurons at frequencies up to 200 Hz and rapidly inactivate, improving neuronal activation efficiency [21]. By extending the channel opening time of ChR2, researchers engineered ChR2 mutants with long-term activation characteristics called step-function opsins (SFO) [10]. Among them, mutation at the C128 site extended the channel opening time of ChR2 by several seconds [22], while mutation at the D156 site extended the channel inactivation time to the minute level [23], and the resulting mutants have been utilized in different studies. The bistable activation step-function opsin (SSFO) developed through chimeric modification based on these two ChR2 mutants has stable properties in both open and inactivated states, enabling control through blue light activation and yellow light deactivation, which facilitates long-term control of neural pathways [24].

In addition to the above mainly blue light-activated microbial opsins, red light-activated opsins—red-shifted opsins—have also attracted researchers' interest. Due to the longer wavelength of red light and better tissue penetration, optogenetic control in deep brain regions becomes more convenient. Meanwhile, the discovery of red-shifted opsins greatly increases the variety of opsins and expands the available opsin toolbox. The red light activation characteristic enables red-shifted opsins to be used in combination with blue light-activated opsins and genetically encoded calcium fluorescent probes (GECIs) to achieve multiple optical controls or simultaneous recordings of neural circuits [10]. VChR1 is a red-shifted opsin found in Volvox that can be activated at 535 nm and causes neuronal depolarization [25]. Mutants C1V1 and ReaChR based on VChR1 modification can achieve maximum activation effects at 539 nm and demonstrate better membrane localization expression capabilities in mammalian neurons [24][26]. Through de novo sequencing of over 100 algal genomes, red-shifted opsins Chrimson and Chronos were also discovered successively. Chrimson can exhibit maximum photocurrent under 585 nm light stimulation, showing longer red-shift characteristics compared to other red-shifted opsins. Chronos can also be activated under yellow or blue light conditions and has fast kinetic properties. The good difference in activation spectra allows Chrimson to be used together

with Chronos to simultaneously activate two independent groups of neurons with red and blue light without mutual interference [27]. Because Chrimson can also be activated at longer wavelengths (720 nm), it demonstrates significant advantages when used in combination with calcium fluorescent probes [21].

2.1.2 Photoinhibitory Opsins

In neurobiological research, neuronal inhibition is equally important for exploring neural circuit functions. Photoinhibitory opsins refer to a class of opsins that can be activated by light and cause neuronal inhibition, among which the most widely used is NpHR opsin from halorhodopsin [28]. NpHR is a chloride ion pump that pumps chloride ions into the cell under light stimulation, causing neuronal hyperpolarization and thus triggering neuronal inhibition. Through structural variation and chimerism of NpHR, researchers engineered the eNpHR3.0 variant that can be broadly activated under green, yellow, or red light conditions [29].

Through expression in cultured hippocampal neurons, eNpHR3.0 was further found to achieve maximum activation photocurrent at 590 nm and optimized membrane localization capability [16]. However, neuronal inhibition achieved through eNpHR3.0 can cause changes in the reversal potential of GABAAR, thereby interfering with neuronal excitability, which has raised considerable questions about its application [30]. In addition, proton pump opsins represented by Arch [31] (an archaeal rhodopsin found in Natronomonas), Mac [32] (found in fungi), and eBR [33] (a rhodopsin protein found in Halobacterium) have also received significant attention. These opsins mainly inhibit neurons by pumping protons out of the cell, causing less interference with GABAergic neuronal excitability and offering greater advantages in rapid deactivation and photocurrent generation rates compared to chloride pump opsins [31].

Similar to photoactivatable opsins, researchers are also committed to developing red light-controlled inhibitory opsins. Mutants eArch3.0, eArchT3.0, and eMac3.0 based on Arch and Mac modifications all have longer wavelength activation characteristics and can be activated under 520-550 nm light control [34]. The recently discovered and optimized Jaws chloride pump opsin in halobacteria has more efficient red-shifted photosensitivity and non-invasive neuronal inhibition characteristics, making Jaws widely applicable in deep brain tissue research [34]. However, red light-controlled opsin activation in neurons is still limited by myelin sheath interference with optical conduction, thus the stability of light source delivery in neural tissue needs improvement [34].

Since ion pumps can only transport single ions under single-photon conditions, their efficacy and stability are inferior to the ion channel opsins mentioned previously. Therefore, researchers engineered the inhibitory chloride channel opsin iC1C2 based on the C1C2 protein structure, which can be activated under blue light and has fast kinetic properties [35]. The development of inhibitory step-function opsins further meets the requirements for long-term neuronal in-

hibition. The inhibitory step-function opsin SwiChR developed based on ChR mutation can trigger long-term inhibition with short-duration light exposure, thereby avoiding the effects of long-duration light exposure on cytotoxicity. The first-generation mutant SwiChRr, in addition to long-term inhibition, can also reversibly and stably open chloride channels like GABAAR chloride channels, enabling fine-tuning of neuronal activity [30]. The second-generation mutant SwiChRr has slower kinetic properties than GABAAR chloride channels, thus meeting the research needs for fine regulation of neuronal activity and interactive behaviors [21][36]. Table 1 provides characteristics of several common optogenetic tools.

Opsin modification and development also focus on exploring membrane localization and subcellular structure targeting. By fusing signal peptides or gene fragments related to organelle localization with opsins, researchers have achieved enriched expression of opsins in subcellular structures [29]. Using eNpHR3.0 modification as an example, fusing the N-terminus of NpHR with the ER export sequence of potassium channel Kir2.1 and inserting the Golgi transport sequence TS internally can greatly enhance eNpHR3.0 membrane localization and photocurrent intensity [29]. Additionally, fusing myosin Va or VI binding domain sequences in ChR2 greatly enhances ChR2 localization in dendritic or axonal membranes [21], while fusing synaptic vesicle protein gene sequences in Arch3 can achieve its expression in presynaptic membranes [37]. The so-CoChR based on the newly discovered photosensitive channel protein CoChR can also be concentrated in neuronal somata [38]. These localization modifications greatly promote the application of opsins in precise neural circuit control. However, subcellular structure localization modifications can lead to reduced opsin expression levels, thereby affecting photocurrent generation. Therefore, enhancing expression levels and localization specificity, as well as improving efficient intracellular light delivery, are future optimization directions.

2.2 Targeted Expression of Opsins

The high diversity and complexity of neural circuit composition make labeling specific cell types or neural projections important for optogenetic research in neurobiology. Currently, opsin expression in specific neural circuits can be achieved mainly through viral vectors containing opsin genes and cell-specific promoters, establishment of transgenic mouse lines, and genetic knock-in techniques [39][40].

First, the simplest and most commonly used method to express opsins in specific neurons is to package gene fragments containing cell-specific promoters and opsins into viral vectors and inject them into specific brain regions to achieve opsin expression through viral infection (Figure 1a [Figure 1: see original paper], Figure 2a [Figure 2: see original paper]) [21]. Commonly used viral vectors include lentivirus, adeno-associated virus (AAV), rabies virus, and herpes simplex virus (HSV) [21]. Lentivirus can achieve long-term stable expression through integration into the cellular genome, but its infection efficiency is low

and unsuitable for direct animal experiments [41]. For adeno-associated virus (AAV), although the expression time is relatively short, its high infection efficiency and large loading capacity make it more widely used in neural circuit research [42]. Rabies virus with retrograde trans-synaptic infection capability and herpes simplex virus with anterograde trans-synaptic infection capability also have broad applications in neural circuit and projection studies [43]. Commonly used neuron-specific promoters include CaMKII (specifically expressed in excitatory neurons of the cerebral cortex), DRD (specifically expressed in nucleus accumbens neurons), and MBP (specifically expressed in oligodendrocytes) [21][44]. When studying the function of a small group of neurons, more specific promoters are needed, such as tyrosine hydroxylase (TH), which can specifically label dopaminergic neurons for functional studies of dopaminergic neurons [45]. Studying the functions of different types of neurons requires finding more specific promoters, and the development of single-cell sequencing technology will greatly promote functional classification of neurons and provide more information for labeling specific neurons. Through optogenetic activation or inhibition of neural circuits, researchers have elucidated the functions of various neural circuits, including the mechanism by which hypothalamic hypocretin-expressing thalamic neurons regulate sleep-wake transitions and the mechanism by which DRD2-expressing nucleus accumbens neurons regulate risk-based decision-making [16][21]. However, because viral vectors can package only limited lengths of specific promoter fragments, this method has limitations in opsin expression in certain neurons.

Using transgenic technology can solve this problem. By establishing transgenic mouse lines that express opsins in specific neural cells, stable opsin expression can be achieved, providing convenience for studying such neurons or neural circuits [32]. The drawbacks of this technology are that it cannot achieve the high spatial accuracy of viral vector infection technology, and secondly, when studying different opsins or different neural circuit functions, it is necessary to re-establish stable transgenic mouse lines, which is not reasonable in terms of operation and time cost.

The genetic knock-in technique perfectly balances the advantages of spatially accurate neuronal expression and simple operation. Taking the recombinase system (Cre-LoxP or Flp-Frt) technology as an example, by constructing mouse lines that express Cre or Flp recombinase in specific neurons, and then packaging recombinase-dependent opsins controlled by universal promoters into viral vectors and injecting them into brain regions of interest, opsin expression in specific neurons can be achieved [32][46] (Figure 1b). Since this strategy greatly reduces the limitation on viral vector loading capacity and has the advantages of high accuracy and simple operation, this technology is most widely used in the field of optogenetics and has made many neural circuit studies possible, such as how parvalbumin-expressing cortical interneurons regulate social behavior [21]. However, this strategy is also limited by some factors, such as the long time required to establish recombinase lines and the lack of specific promoters for certain groups of neurons to be studied, making it impossible to precisely locate

specific types of neurons and tissues. In summary, the strategy of using genetic knock-in technology to achieve neuron-specific opsin expression will be more widely applied with technological improvements.

Another important aspect of neural circuit research is studying how projection relationships between different brain regions control behavior. The current research difficulty mainly focuses on how to visualize functional connections between brain regions using opsins. Theoretically, using light to directly stimulate opsin-expressing projection nerve fibers can achieve activation of downstream brain regions, but this method requires several weeks to express sufficient opsins in long-projection nerve fibers and needs to be based on pre-explored projection relationships between brain regions [21]. Additionally, sparse-combinatorial labeling of neurons can be used to explore projection relationships between brain regions, but this method has certain blindness [47]. Therefore, scientists further developed trans-axonal retrograde infection viruses, among which Canine Adenovirus (CAV) is one. Suppose we have a mouse line that stably expresses Cre recombinase in brain region X. When CAV viral vectors containing Cre-dependent opsins are injected into brain region X, the opsin gene will be recombined under the action of Cre recombinase to achieve expression in brain region X. Then, through CAV virus replication and packaging of opsin particles, retrograde trans-synaptic infection of upstream brain region Y of Cre neurons will result in opsin expression in the somata of region Y, thereby enabling activation of this projection circuit through optical stimulation of neuronal somata in upstream brain region Y [48][49] (Figure 2b). In addition, efficient rabies virus also has retrograde trans-synaptic infection characteristics and is therefore commonly used to study functional connections between brain regions. However, due to the strong neurotoxicity of rabies virus, its application is limited. The newly developed CVS-N2cΔG rabies virus strain has lower neurotoxicity, expanding the application of rabies virus in optogenetics [50]. Genetically edited pseudorabies virus from the Herpesviridae family can also be used for retrograde axonal labeling, but its lethal infectivity to experimental animals limits its widespread use [51].

Compared with the wide application of retrograde trans-synaptic infection viruses in neurobiology, the development of anterograde trans-synaptic infection viruses lags far behind. Herpes simplex virus H129 strain [52] and vesicular stomatitis virus [53] are currently the main anterograde trans-synaptic infection viral vectors under investigation, but their cytotoxicity limits further application [21]. Recent studies have also found that adeno-associated viruses (AAV), including AAV1 and AAV9, also have anterograde trans-synaptic infection characteristics [54]. Therefore, efficient anterograde tracing viruses need further development and modification.

2.3 Optical Transmission Technology

Optical transmission technology mainly includes light sources and transmission technologies that deliver light sources to tissues. Laser (diode or diode-pumped

solid state, DPSS) and LED (light-emitting diodes) are two commonly used light sources [55].

The main advantage of lasers is the very narrow spectral range (<1 nm), which reduces cross-interference in multicolor optogenetics and imaging. Another advantage is low divergence, which greatly improves the efficiency of fiber coupling for transmission. However, the main disadvantages are high cost and limited stimulation frequency in certain wavelength bands [16]. LED light sources do not require complex electronic control components, have lower cost, have greater potential for developing wireless transmission, and can easily achieve high-frequency stimulation. However, the main disadvantages are the wider wavelength range and greater divergence, which limits high-intensity light transmission [55].

Currently, the most commonly used transmission method is fiber optic transmission. The optical fibers used in fiber optic transmission typically have diameters of tens to hundreds of micrometers. By implanting them into target brain regions and coupling them with light sources, light input can be achieved in freely moving animals. When the input light intensity (depending on the type of opsin used and its expression level) exceeds the threshold required for opsin activation, the opsin can be activated to cause neuronal activation or inhibition. The light intensity required to trigger action potentials in ChR2 is generally 5 mW/mm^2 [15]. Given the known light intensity required at the fiber tip, due to scattering and absorption by surrounding brain tissue, the light intensity around the fiber tip gradually decreases with distance. Therefore, fiber implantation technology can only activate opsins within a certain region and cannot achieve uniform light intensity in surrounding areas. Although stronger light intensity increases the effective activation range, light-induced tissue heating can damage neurons [56]. Therefore, in addition to appropriate light intensity and control experiments, reducing light intensity attenuation and tissue thermal damage are future development directions for fiber optic transmission.

Fiber optic technology is now widely used in deep brain stimulation experiments in freely moving animals, but fiber implantation inevitably causes a certain degree of brain tissue damage and local bleeding, especially when using large-diameter fibers for high-intensity light input [57]. Therefore, for light stimulation requiring larger brain regions or special spatial positions with multiple stimulation points, researchers have developed miniaturized microfibers and tapered fibers [58] to reduce mechanical damage to tissues. Meanwhile, fiber bundles containing hundreds or thousands of microfibers can be packaged in an insulated cannula and implanted into target brain regions to achieve simultaneous stimulation of a larger brain region or sequential stimulation of different brain regions [59]. Another experimental scheme for multi-point brain region stimulation is to attach a silicon chip to the implanted fiber end and couple it with a light source array [60]. In summary, these diverse optical transmission strategies make optical input in multiple brain regions and spatial positions more convenient and effective.

3 Integration of Optogenetics with Other Technologies

After activating target neurons or brain regions, recording how neurons respond and their response patterns is also important for studying the causal relationship between neural circuits and behavior. Currently, optogenetic technology is mainly combined with tools or techniques for recording neuronal activity, such as functional magnetic resonance imaging (fMRI), calcium imaging, and electrophysiological techniques [21]. Combining optogenetics with fMRI, a whole-brain activity recording technology, enables the study of activation in different structural regions of the brain, but the disadvantage of this method is poor temporal resolution, making it difficult to use for fast-responding neural circuits and freely moving animals [61]. Electrophysiology is the gold standard for recording neuronal activity, allowing precise stimulation or recording of a single neuron' s activity, but its disadvantages are low throughput and strong invasiveness, which have certain effects on neuronal activity. The combination of genetically encoded fluorescent probes with optogenetics can overcome these limitations. For example, genetically encoded calcium indicators (GECI) and genetically encoded voltage-gated probes can achieve neuronal activity recording after opsin activation under single-photon stimulation, helping to elucidate the effects of circuit function and information interaction between neurons on behavior. In the latest research, the combination of the new photosensitive protein soCoChR with two-photon computer-generated holography (CGH) successfully achieved optical manipulation of single neurons and compressed the response time to within 1 millisecond, thereby greatly improving the precision of optogenetic technology in exploring causal relationships between specific neural circuits and brain functions. CGH technology also supports 3D imaging, making optical control potentially applicable to complex three-dimensional models [62].

As one of the most influential technologies in neurobiology over the past decade, optogenetics has also demonstrated great potential in clinical disease research. Studies in Parkinson' s disease mouse models (with drug-induced dopaminergic neuron destruction) have shown that after transplanting opsin-expressing human dopaminergic neurons into the mouse striatum, optical activation of these dopaminergic neurons achieved symptom recovery from hemiplegia and paw tremor in mice, thereby confirming the role of dopamine molecules in Parkinson' s disease and preliminarily testing the feasibility of using optogenetic technology to treat Parkinson' s disease [5]. Through optogenetic inhibition of abnormally discharging neurons, preliminary prevention of epileptic seizures was achieved in epilepsy mice [6], and optical control was also used to achieve wing flapping in headless Drosophila [12]. Additionally, optogenetics has yielded preliminary results in studies of disease models such as depression, schizophrenia, autism, and drug addiction [63]. With further development of opsin characteristics and diversity, optogenetic technology is expected to be applied to primate models such as rhesus monkeys that are more closely related to humans [6]. Optogenetics has recently been attempted as a novel pacemaker in arrhythmia disease models to control rhythm and contraction strength as a compensatory treat-

ment for cases unresponsive to conventional pacemaker therapy, and has also been attempted to restore tendon contractility in skeletal muscle tendon diseases [64][65]. Thus, optogenetic technology has great application potential in both pathophysiological mechanism research and disease treatment.

However, optogenetic technology still has many problems that limit its further development. Taking opsin expression as an example, differences in opsin expression levels among similar neurons can lead to different effects under the same light intensity stimulation, causing deviations in integrated feedback in neural circuits from physiological conditions [66]. The photosensitivity of opsins causes synchronous activation or inhibition of opsin-expressing neurons, thereby suppressing temporal sequential neuronal interactive behaviors and the intrinsic response characteristics of neurons [67]. Although combined use with GECI probe technology can achieve localization of activation sequences of neurons in neural circuits, synchronous activation masks multidimensional neuronal interactions and early fast-circuit behaviors [10]. In terms of using viral vectors for opsin expression, although recombinase systems can achieve neuron-specific expression, control of site-specific expression in different brain regions and uniformity of expression stability still requires minimizing interference with the cell' s own material expression while increasing opsin expression. Additionally, the thermal effects of light sources and direct stimulation of neurons require more improvements to reduce invasiveness to brain tissue and better simulate physiological situations. The application of optogenetic technology in disease research is also limited by disease model construction. How to quickly and stably construct mammalian disease models that conform to physiological conditions will continue to be a research hotspot in the field.

In summary, optogenetic technology will continue to develop rapidly, and the application of this technology will help us answer more mysteries in neurobiology and problems in disease treatment.

Figure 1 [Figure 1: see original paper] Opsin targeting expression technology [21]

Figure 2 [Figure 2: see original paper] Opsin expression and activation in mouse brain regions [21]

Table 1 Characteristics of common optogenetic tools

Opsin	Ion Selectivity	Activation/Inhibition	Activation Spectrum (nm)	Notes
ChETA	Cation channel	Activation	470	

Opsin	Ion Selectivity	Activation/Inhibition	Activation Spectrum (nm)	Notes
SSFO[1]	Cation channel	Activation	470/590	[1]Activated by brief 470nm blue light, maintains for 30min, deactivated by 590nm red light
Chrimson[2]	Cation channel	Activation	585/720	[2]Can be activated at two wavelengths, maximum signal at 585nm, usable with calcium probes at 720nm
NpHR	Chloride pump	Inhibition	590	
iC1C2	Chloride channel	Inhibition	475	

Opsin	Ion Selectivity	Activation/Inhibition	Activation Spectrum (nm)	Notes
SwiChR[3]	Chloride channel	Inhibition	475/632	[3]Activated by brief 475nm blue light, deactivated by 632nm red light

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